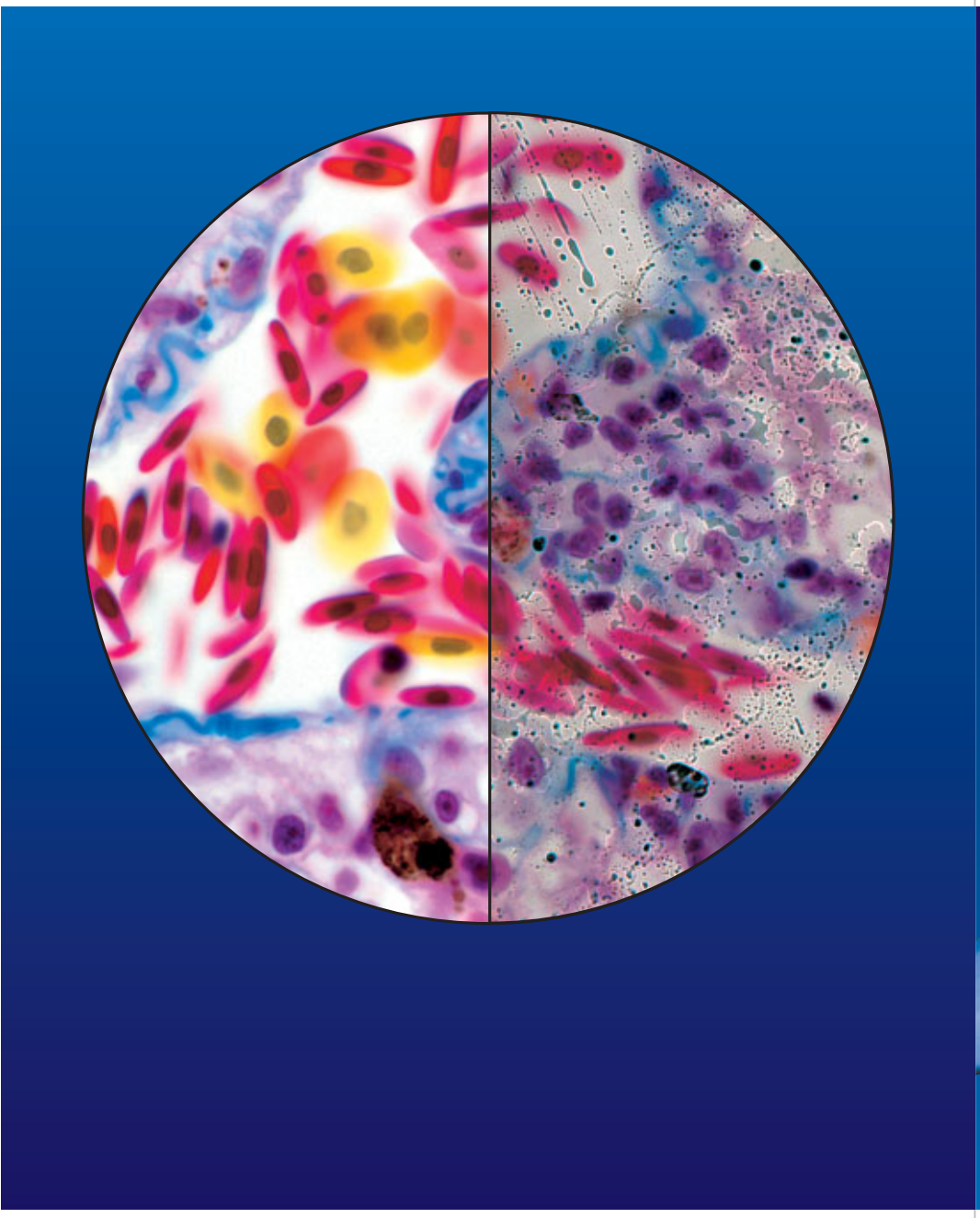
Principles

The Clean Microscope



Recognizing dirt and removing it correctly

ZEISS

We make it visible.

Clean microscope optics are a prerequisite for successful microscopy and perfect images.

Over the years a variety of cleaning procedures have been recommended. Many users remain unsure as to which of these will yield the best results.

The choice of the best cleaning method depends on the nature of the optical surface and the type of dirt to be removed.

Please contact the local Carl Zeiss representative for questions regarding maintenance and service.

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The Effect of Dirt on the Image

The closer dirt is to the object or to the camera sensor, the greater its effect on the visual or photographed image. The critical areas are the following:

1. The external surface of the front lens of the objective
2. The surface of the camera sensor and its protective glass cover
3. Both surfaces of the cover slip
4. The surface of the microscope slide
5. The surface of the camera adapter optics
6. Surface of the upper lens of the condenser
7. The outer and inner surfaces of the eyelens of the eyepiece as well as the upper surface of the graticule
8. The outer surface of the protective glass covering the opening through which light exits
9. Other glass surfaces in the light path e.g. bulbs of halogen- or high pressure lights, fluorescence filters and beam splitters, collector lenses, contrast and heat filters.

Some optical surfaces are more sensitive to dirt than others. The front lens of the objective is particularly critical and is therefore discussed in greater detail below:
For any dry objective, the smaller the free working distance and the smaller the surface area of the concave front lens, the greater the danger of soiling the front lens with embedding media, immersion liquids or dust.
Examples of such objectives are the EC Plan-Neofluar 40x/0.75, EC Plan-Neofluar 63x/0.95 Korr, Achroplan 63x/0.80, 63x/0.95 o.D., Fluar 20x/0.75, Planapo 20x/0.80, Planapo 40x/0.95 Korr, all dry objectives of the type Epiplan and EC Epiplan-Neofluar as well as EC Epiplan-Apo-Objectives with magnifications of 20x, 50x and 100x.

When working with inverted microscopes, the front lens of every objective will be more exposed to dust than that in an upright microscope; all LD dry objectives with magnifications of 32x, 40x and 63x need to be regularly checked.
The front lens of an immersion objective should be cleaned to remove residue both after use and additionally, before applying fresh immersion liquid. The mixing of different immersion media, as well as different lots of the same medium e.g. the immersion oil IMMERSOL F™, can result in blurred images.

The cameras are always to be handled with the utmost care and protected from dirt using all available methods.

Before every critical use, check the front lens of the objective for dirt.

Title:
Toad, liver, stained with Azan.
Planapo 63/1.4. Bright field

How to Recognize Dirt

Familiarity with the best possible results obtainable with a specific technique and procedure permits a user to recognize the consequences of dirty optics. The ability to compare the expected image and the image actually obtained with respect to optimal sharpness, contrast and the absence of contamination-dependent visual artefacts, allows the user to immediately recognize when the microscope is dirty or not.

If the image sharpness or contrast is not optimal, then there is a high probability that the microscope optics are not clean.

In order to determine the location of the dirt, please proceed as follows:

Carefully rotate objectives and cameras a small amount within their thread.

Check the slide and cover slip by moving the specimen while focusing initially on the upper and then the lower surfaces.

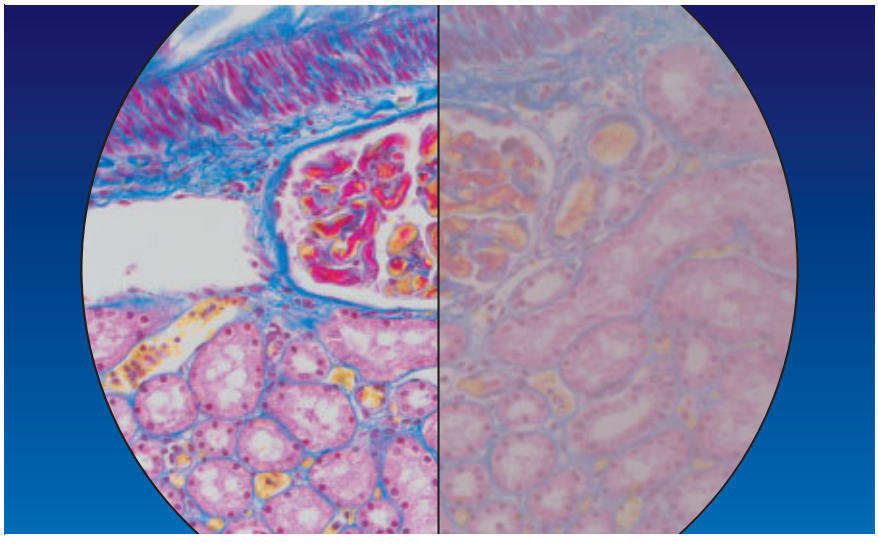
Check the condenser while moving it up and down and if applicable, by swiveling the front lens slightly.

The affected optical surface is identified when a suspected optical component is moved and the dirt follows this movement. The single exception to this rule is the camera: dirt located within the camera will not move when the camera is moved!

A macroscopic check for larger dust particles and scratches on optical surfaces can be carried out using either a magnifying glass (with a magnification of 3–6x) or an eyepiece held the wrong way round.

A soiled objective front lens is easily determined by looking at an evenly lit surface through the wrong end of the objective: The internal lens organization produces an enlarged image of the smallest contaminants present on the external surface of the front lens.

The final check should always involve an assessment of the achieved improvement in image quality.



Clean (left) and oil contaminated (right) objective front lens. Toad, kidney, stained with Trichrome. Planapo 20/0.80. Bright field

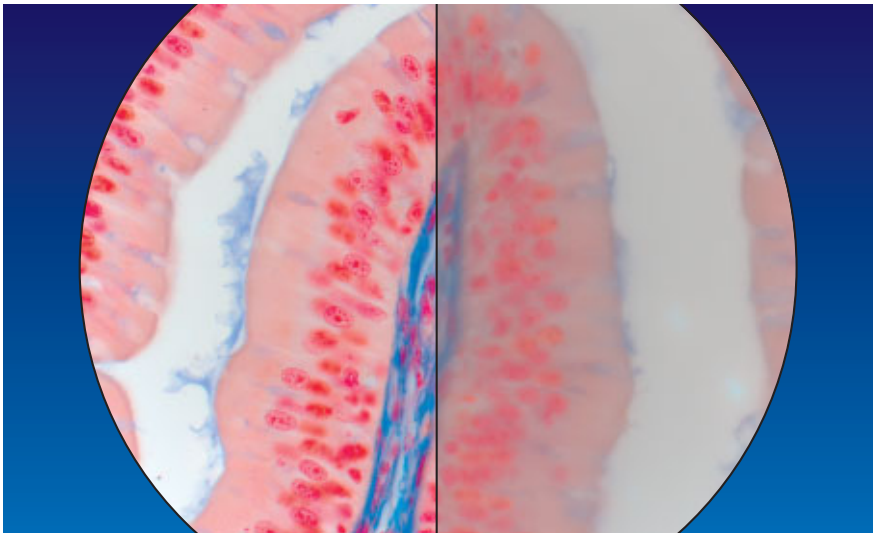
Different Types of Dirt

It is crucial to differentiate between dust (e.g. glass dust from slides, flakes of skin from the microscopist, fluff from clothing, pollen from spring and summer flowering) and other dirt (e.g. liquid or dried-out embedding or immersion media, culture solutions, residue from improper cleaning attempts, fingerprints and grease).

Dust particles can either rest loosely or can be more or less stuck to optical surfaces. Other dirt is either water-soluble or may only be completely removed using organic solvents.

A blurred image
may not always be due to dirt:
Using an objective with a large numerical aperture in conjunction with a cover slip of the wrong thickness can result in blurred images (spherical aberration).

Dry objectives of this type normally have a correction collar, which permits compensation for the spherical aberration:
Turn the correction collar until the optimal image contrast and sharpness is achieved while continuously adjusting focus.
Many highly corrected immersion objectives also require specially selected cover slips with a thickness of 0.17 mm, if the best image is required.
Immersion objectives should only be used in conjunction with a suitable, bubble-free immersion oil.
Oil immersion objectives should only used with IMMERSOL™ from Carl Zeiss; the water immersion objective, C-APOCHROMAT, ideally with only distilled water or IMMERSOL W™.
The occasional recommended use of anisole as an immersion medium results in loss of sharpness and contrast. Anisole can attack the cement of front lenses, especially that of older objectives.



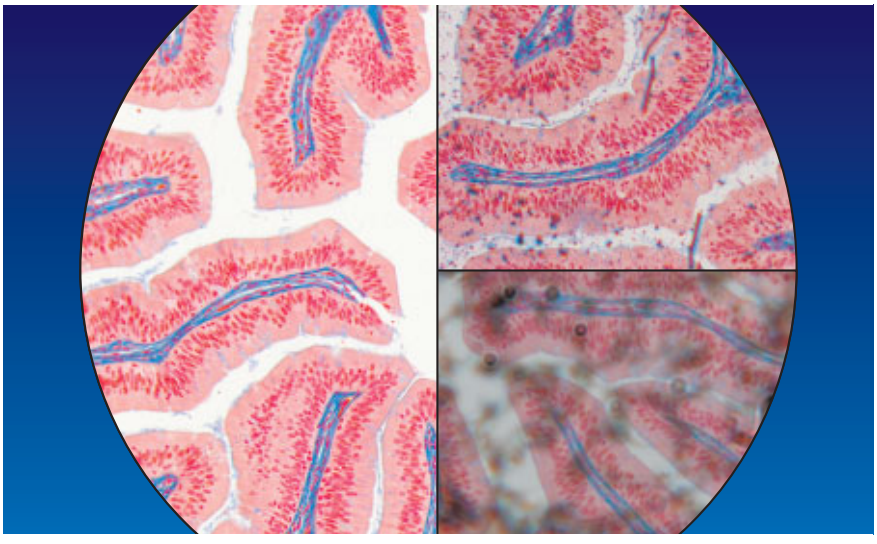
An imperfect image despite clean optics caused by spherical aberration: Correction collar of the planapo 40/0.95 objective correctly (left) and incorrectly adjusted (right). Frog, small intestine, stained with Azan.

Different Optical Surfaces

Concave or convex surfaces (e.g. front lens of dry objectives and condensers, the eye-lens of some eyepieces) should be distinguished from planar parallel or flat surfaces (e.g. the front lens of most of the immersion objectives and condensers, filters, the protective glass covering camera sensors or the opening through which light exits). Concave or convex surfaces are cleaned using either the cotton or the new polyester swabs as described on page 6.
Easily accessible flat surfaces may be similarly cleaned or with soft disposable cellulose wipes.

Microscope optics can be composed either of optical glass, quartz or polymers. The upper surface of almost all will be coated to minimize stray light. Some anti-reflex coatings may be wipeable (e.g. the eyelens of eyepieces) or not, due to their softness. Generally, anti-reflex coatings are composed of magnesium fluoride and should only be cleaned with agents free from ammonia and acid. Sometimes household glass cleaning agents, which contain dilute ammonia (e.g. SIDOLIN, SPARKLE, Blue WINDEX), are recommended but they should not be used routinely.

Some optical components are surrounded by black anti-reflex surfaces, which are sensitive to organic solvents. The plastic and rubber parts of the eyepiece will likewise be attacked by organic solvents (e.g. acetone, chloroform).
In older microscopes, lenses are cemented using an alcohol soluble cement such as Canada balsam. These days the lens cement is generally a polyacrylic synthetic resin, which does not have this problem.
The internal workings of the microscope – the optical surfaces, components of the fluorescent filter sets, cameras and camera adapters should never be cleaned by the user, but by experienced customer representatives from the original manufacturer.
The user should only clean: the external surface of the objective front lens, the condenser front lens, the eyepiece eyelens, glass color- and conversion filters, and the external surface of the protective glass covering the opening through which light exits.



Dust on the cover of the opening through which light exits (upper right), extremely dirty camera (bottom right) and clean optics (left).
Frog, small intestine, stained with Azan. Planapo 10/0.45. Bright field.

Cleaning Agents and Procedures

The goal is to completely remove dust and dirt without leaving any residue of the cleaning agent or damaging the surfaces.

The following equipment is required:

– Long, thin wooden sticks, preferably of bamboo (obtainable from Chinese restaurant suppliers) or a comparable not too flexible material.

– High purity cotton (e.g. that used in ophthalmology supplied by KERMA, Germany) or WHATMAN lens cleaning tissue 105.

– Absorbent polyester swabs for cleaning optical components.
ITW Texwipe CleanTips® swabs (TexWipe) represent a very good alternative to the cotton swabs and can be re-used.

– Soft cosmetic cellulose tissue (e.g. Kim Wipes soft, KLEENEX).

– Dust blower (laboratory suppliers, pharmacies).

– Distilled water.

– Freshly prepared solution of 5–10 drops of a washing-up liquid (e.g. Fairy Ultra, Fit) in 10 ml distilled water.

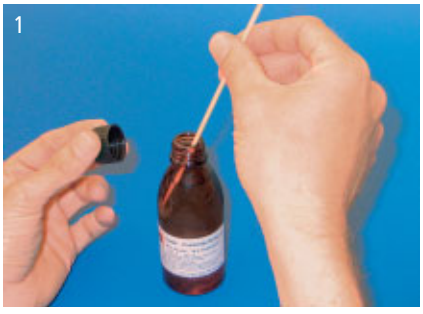
– Solvent for the removal of greasy or oily dirt, such as the Optical Cleaning Solution L (recipe from Carl Zeiss), pure petroleum ether (analytically pure, boiling point <44°C) or, exclusively for cleaning cover slips, pure acetone.

For the easy cleaning of flat surfaces (e.g. the removal of immersion media from cover slips or the front lenses of immersion objectives), soft tissue (e.g. Kleenex) soaked in diluted washing-up liquid is suitable.

Care: The smooth lens paper (so-called Joseph paper), which is often readily available in research laboratories, is not for cleaning but is intended only for the dust-free storage and protection of optical components. For cleaning purposes this lens paper is too harsh; it also doesn't absorb the dirt effectively or quickly enough. WHATMAN Lens Cleaning Tissue 105 represents the single exception.
For the cleaning of all other optical surfaces either freshly made cotton swabs or the new polyester swabs, ITW Texwipe Clean Tips®, are used.

Preparation of cotton swabs

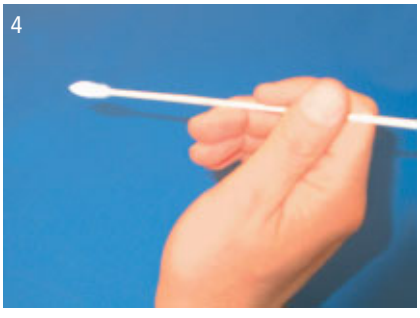
- Wash hands (powdered, latex gloves are not suitable).
- Dip the stick into the cleaning solution (aqueous- or organic solvent). The cotton fibers attach better to the stick as a result.



- Dab the stick onto the wad of cotton and loosen some fibers. Do not compact the cotton otherwise the fibers will not separate easily.
- Turn the stick so that an even, elliptical cotton bud forms at the tip.

- To protect the cotton tip from dirt, the stick should be stored in a polythene bag. It should not be handled as perspiration and grease, from the fingers of the users, will significantly affect its ability to clean.

- Remove the cotton tip after every wipe and replace it with a fresh cotton bud.
- The stick can be used for a long period of time. Use separate sticks for water-based solutions and organic solvents.



If the use of WHATMAN Lens Cleaning Tissue 105 is preferred, fold the sheet around the stick so that a sharp point is generated. The point should not be handled. Use the tissue only once and then replace it.

The polyester swabs, ITW Texwipe CleanTips®, can be used until they no longer clean well.

Cleaning Procedure

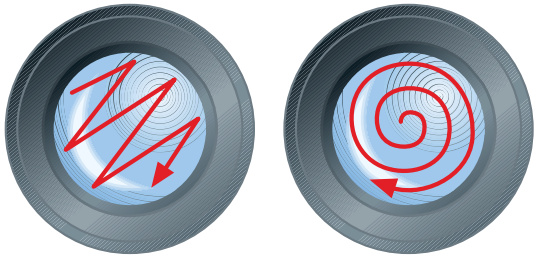
1. Blow all loose dust particles away with a dust blower.
2. Remove all water-soluble dirt with distilled water. If this is unsuccessful repeat using a solution of diluted washing-up liquid. Remove any remaining residue with a dry cotton swab, but breathe on the surface first to generate a film of moisture. In so doing, be careful not spray droplets of saliva.
3. To remove **oily** dirt, use a solution of dilute washing-up liquid initially. If this does not produce a satisfactory result, repeat the cleaning using a solvent (Optical Cleaning Solution L, petroleum ether).
4. **Greasy** dirt must always be removed using a solvent.
5. After cleaning check the surface (see the section "How to recognize dirt").

Place the objectives, eyepieces and cameras on a dust-free surface (e.g. fresh aluminum foil). All other optical components to be cleaned should be as accessible as possible.

Dip the cotton swab or the ITW Texwipe CleanTips® swab into the cleaning solution and shake off excess liquid. An excess of liquid in a cotton bud will flow over the rim of the lens and attack the lens cement. This may consequently lead to the removal of the cement between bonded components. The solvent should remove as much dirt as possible. In order to increase the retention time of volatile organic solvents in the cotton bud, some users chill the solvent (–10°C to –20°C). Chilled solvents have a disadvantage: due to their low temperature, condensation may form on the lens surface and leave a residue. A more suitable way to improve the retention time of a solvent is to add isopropanol, for example.

Cleaning is achieved using a spiral motion **from the center to the rim.** Never wipe using zig-zag movements as this will only spread the dirt.
With larger optical surfaces (e.g. tube lenses) the spiral motion **starts initially at the rim** before moving to the middle and is only then followed by a center to rim cleaning motion.
Normally several spiral wipes are recommended.

We recommend pure, volatile petroleum ether or the Optical Cleaning Solution L from Carl Zeiss.



**Wipe using a spiral movement –
do not use a zig-zag motion!**

Not all solutions can be recommended to clean the optics of a microscope. Some clean very effectively, but are either toxic (e.g. chloroform, acetone) or environmentally unfriendly (e.g. Freon®, tetra-hydrochloride); others will leave surface residue (e.g. xylene, toluene, diethylether).

Residue forms particularly with the use of xylene and absolute ethanol, and above all when the dirt contains water-soluble components.

Acetone can be recommended when oil and grease are to be removed from cover slips. Acetone attacks most types of plastic as well as rubber, and as a consequence its use to clean eyepieces can be problematic, for example. It cannot be excluded that acetone may attack cemented optical components (e.g. objectives, TV-adapters, eyepieces) when used frequently.
Acetone can also dissolve specialized organic coatings.

How Can Contamination be Avoided?

The opening of the binocular tubes should always be protected either with the eyepieces or with dust covers. If no dust covers from the manufacturers are available, aluminum foil is a suitable substitute. The best fundamental method to prevent **dust accumulation** is to first cover the microscope with two additional plastic bags and then the dust cover supplied by the manufacturer.
In tropical regions this measure is not recommended as it can frequently lead to a build up of fungus.
Fungal contamination can best be minimized by reducing the humidity of the room either by air conditioning or by installing an infrared lamp above the microscope (at a minimum distance of 150 cm or 5 Feet). Fungal contamination is almost impossible to remove.
The microscope should never be located in a position where it could be affected by **acidic or alkaline vapors,** such as in or near a wet chemical photographic laboratory.

Cleaning External Microscope Components

The painted surfaces of microscopes from the AXIO range are powder coated and extremely durable. They can be cleaned well with a very lightly moistened microfiber cloth. Loose dust and other dirt can be removed using a brush of soft hair used exclusively for this purpose.

Together with the cleanliness of the microscope optics, perfect sample preparation is the deciding factor for optimal results:
e.g. the thickness of the histological section, staining intensity, the refractive index and dispersion of the embedding media and immersion fluids, and when performing high resolution microscopy – the distance of a living cell from the cover slip.

A thin and clean preparation of the fresh water protozoan, Dimorpha mutans. Planapo 63/1.4 Phase contrast.

What to Watch Out for When Cleaning Microscope Optics!

1. When starting to clean, don't forget to use a dust blower except when fluids (such as immersion oil) are to be removed.
2. Never wipe lenses with dry swabs or tissue – this causes scratches!
3. Do not use abrasive materials e.g. leather wipes, dry linen cloths or polystyrene sticks as recommended by some manufacturers.
4. Do not apply any solvents before trying distilled water (a film of distilled water can be generated by breathing on the surface), except when grease is to be removed.
5. Do not use ethanol or acetone for the cleaning of older microscopes (e.g. the STANDARD range from Carl Zeiss Oberkochen or the MIKROVAL- and JENA-Microscopes 250 CF range from Carl Zeiss Jena).
6. Do not use any disposable cotton swabs (e.g. Q-Tip®) instead of the described cotton or ITW Texwipe CleanTips® swabs, as the former are not free from contamination.
7. Beginners should not use any of the occasionally recommended metal rods instead of the wooden (bamboo) sticks, as the front lenses may be more easily damaged.
8. Do not use any of the optical spray cans containing pressurized liquid air. The pressurized air from these sprays leaves a slight, but difficult to remove, residue.
9. Never use acids or ammonia to clean objective front lenses.
10. Never try to clean the internal optical surfaces, cameras or adaptor optics.

Suppliers and Recipes

KERMA cotton N 1. DAB 6.
The cotton used in ophthalmology is 100 % pure cotton (DIN 61 640-A, Ph. Eur., DAB). It is absolutely pure, highly absorbent and soft. The fibers can be blown away from optical surfaces.
www.sbh-hainichen.de/kerma/prod/spez2.htm

WHATMAN Lens Cleaning Tissue 105
Lens paper in folders 10 cm x 15 cm,
25 blocks each with 25 sheets, Order Nr. 2105 841.

The only lens tissue recommended by Carl Zeiss. It is chemically pure, silicon free and contains absolutely no additives.
This product is also supplied by other companies e.g. KODAK
www.whatman.plc.uk

Absorbent polyester swabs for cleaning optical components ITW Texwipe CleanTips®
(Alpha®-, Clean Foam®- or Absorbond® series.)
Obtainable in different sizes and absorption abilities e.g. from the company Basan under TEXWIPE TX743B.
www.texwipe.com **www.basan.com**

Recipe for Optical Cleaning Solution L from Carl Zeiss
Recipe 85 % petroleum ether, 15 % isopropanol.
(The solution is not sold by Carl Zeiss.)

The petroleum ether (also known as benzine or rubbing alcohol) should be analytically pure and have the lowest possible boiling point (≤ 44°C). Heavier benzene fractions are not suitable as they leave an insoluble residue on the optical surface.
Acetone that is recommended exclusively for the occasional cleaning of oil contaminated cover slips should also be analytically pure.

Safety advice
When working with chemicals, solvents and other possible hazards, please be sure to follow the current, country-specific, safety regulations.

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